REMARKS

Interview

Applicants thank Examiners Davis and Le for the courtesy extended at the interview held May 4, 2004, and here confirm the substance of the Interview Summary Record.

Status of and amendment to the claims

Claims 6 - 14 are pending.

Claims 1 - 5 and 15 - 26, previously withdrawn pursuant to restriction requirement, are canceled herein. Claim 8 is canceled herein without prejudice.

Claims 6, 7, and 9 - 14 are thus presented for further examination.

Claim 6 is amended herein more particularly to point out and distinctly claim applicants' invention, with consequential amendments to dependent claims 7, 10 and 12. No new matter has been added.

Support for the retentate chromatographic method of step (a),[1] newly added to claim 6 by amendment herein, can be found particularly in original claim 7; and in the specification at page 70, line 13 - p. 71, line 9. Affinity capture probes *per se* are defined explicitly at p. 38, line 25 - p. 39, line 8 and at p. 65, line 30 - p. 67, line 23.

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[1] ". . . (a) in parallel, adsorbing a subset of proteins from each of two complex biologic samples to an adsorptive surface of an affinity capture probe . . . "

Support for laser desorption ionization (LDI) mass spectrometry is found throughout the specification, including the description of a preferred embodiment of an affinity capture probe QqTOF orthogonal extraction tandem mass spectrometer at p. 46, line 3 - p. 65, line 29 and in FIGS. 1 and 2.

Support for "identity ***candidates***", as newly recited in claim 6, step (d), can be found, *e.g.*, in claim 2 as filed and in claim 7 as filed; in the specification at p. 15, lines 14 - 16, lines 23 - 25, and lines 28 - 31; p. 16, lines 1 - 9; p. 16, line 25 - p. 17, line 2; p. 20, line 19 - p. 22, line 5; in Example 4 ("Differential Peptide Display for Quick Protein identification ('QPID')", specification pp. 122 *et seq.*; and elsewhere throughout the specification.

Support for the final clause of claim 6, " whereby the correlation [effected in step (e)] identifies a protein ***as had been differentially present*** in the two complex biologic samples," can be found particularly in Example 1, specification pp. 111 *et seq.*, and in Example 4, specification pp. 122 *et seq.*, as further discussed below.

Applicants' Response to Obviousness Rejections

Claims 6 and 8 - 14 stand rejected under 35 U.S.C. § 103(a) as having been obvious over Hutchens *et al.* (WO 98/59362) ("Hutchens") in view of Dongre *et al.* (TIBTECH, vol. 15, October 1997) ("Dongre"). Claims 6 and 8 - 14 stand rejected under 35 U.S.C. § 103(a) as having been obvious over Liebler *et al.* (U.S. Pat. No. 6,379,970) ("Liebler") in view of Dongre. Claim 7 stands rejected under 35 U.S.C. § 103(a) as

having been obvious over Hutchens in view of Dongre and further in view of Little *et al*. (U.S. Pat. No. 6,322,970) ("Little").

Applicants respectfully traverse these rejections.

The methods of the present invention permit the rapid detection and identification of protein biomarkers, proteins whose presence, or whose physicochemical characteristics, differ as between a control and a sample of interest; as recited in claim 14, such controls and samples can, for example, be "(1) a sample from a healthy source and a sample from a diseased source, (2) a sample from a test model exposed to a toxic compound and a sample from a test model not exposed to the toxic compound or (3) a sample from a subject that responds to a drug and a sample from a subject that does not respond to the drug." Example 1 of applicants' specification details the identification of a protein biomarker that may prove clinically useful in distinguishing prostatic adenocarcinoma from benign prostatic hypertrophy (BPH) (specification pp. 111 - 113); Example 4 of applicants' specification describes the identification of proteins that may serve as biomarkers of tumor hypoxia (specification pp. 122 - 125).

As clarified by amendment herein, applicants' methods comprise the following steps:

(a) in parallel, adsorbing a subset of proteins from each of two complex biologic samples to an adsorptive surface of an affinity capture probe;

(b) detecting at least one protein that is differentially displayed in the laser desorption

ionization (LDI) mass spectra of the two adsorbed subsets;

(c) cleaving proteins adsorbed to said probes into protein cleavage products and detecting protein cleavage products that are differentially displayed in the LDI mass spectra of the two cleaved adsorbed subsets;

(d) determining at least one identity candidate for at least one differentially displayed protein cleavage product with a tandem mass spectrometer; and

(e) correlating the at least one identity candidate for the at least one differentially displayed protein cleavage product of step (d) with a differentially displayed protein of step (b).

The use of laser desorption/ionization mass spectrometry to discover protein biomarkers is not new.

Hutchens, which is commonly owned with the present application, discloses the use of laser desorption/ionization (LDI) mass spectrometry to detect and characterize proteins that are differentially expressed as between biological samples (see in particular Hutchens p. 63, line 1 - p. 64, line 10). Hutchens discloses the advantages in such biomarker discovery efforts of using the selective chromatographic retention of proteins on an adsorptive surface of a laser desorption/ionization probe.

But as the Examiner notes in the outstanding office action, "Hutchens does not teach utilizing the method with tandem mass spectrometry. . . . "

Dongre reviews art-recognized methods for using tandem mass spectrometric data, coupled with database mining, to identify candidates for a protein's identity. The Examiner suggests on that basis that "[i]t would have been obvious to one of ordinary skill in the art to modify the reference of . . . Hutchens to include tandem mass spectrometry."[2]

But even if such combination were motivated or suggested by the prior art, which applicants respectfully traverse, the combination nonetheless lacks correlation step (e) of applicants' claimed methods, vitiating the Examiner's *prima facie* case.[3]

And lacking such correlation step, such combination method would in practice fall well short of applicants' claimed invention. The problem is perhaps best illustrated by applicants' Example 1.

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[2] Liebler omits applicant's steps (a) and (b), beginning the analysis with proteolytic digestion ("the method of the present invention includes digesting the proteins in two samples to a mixture of peptides ***and then comparing*** the abundances of specific peptides") (emphasis added).

Little, similarly, discloses "[a] process . . . for determining the identity of a target polypeptide by comparing the masses of defined peptide fragments of the target polypeptide with the masses of corresponding peptide fragments of a known polypeptide."

[3] "[T]he prior art reference (or references when combined) must teach or suggest ***all the claim limitations***." MPEP (8th ed., rev. 1) § 2143 (emphasis added).

After proteolytic cleavage of a potential prostate cancer biomarker that had been adsorptively purified on a laser desorption/ionization probe, the "resultant peptide signals were submitted for protein database analysis and a preliminary identification of human semenogellin I was made."

Had the analysis *ended* there, after a first phase of mass analysis, as in Hutchens, the biomarker would have been identified as semenogellin I.

And that identification would have been wrong: as further described in applicants' Example 1, the biomarker is in fact seminal basic protein (SBP), a proteolytically derived fragment of semenogellin I.

To make that determination, applicants used tandem MS analysis to provide a list of possible identity candidates -- not unlike Dongre. Critically, however, applicants then tested these database-provided candidates for biological relevance by back-correlating the bioinformatically provided identity candidates to the 5751 Dalton molecular weight of the biomarker as it had been differentially displayed in the initial protein mass spectrum. At 52 kDal, semenogellin I could not possibly have been the biologically relevant form of the biomarker; only the proteolytic cleavage fragment known as seminal basic protein, among the identity candidates, had a mass that matched the protein biomarker as had been differentially displayed in the initial spectrum.

Conversely, had the analysis *started* only with the proteolytic cleavage products, without antecedent analysis of the differentially displayed biomarker itself, as in Liebler,

there would also have been no way to know which, among the identity candidates, was the biologically relevant form.

The correlation called for in step (e) of applicants' claimed methods serves to identify a "protein ***as had been differentially present*** in the two complex biologic samples," and thus serves to provide a biologically relevant answer.

Proteolytic cleavage is but one of the biologic phenomena that would confound the approach advocated by the Examiner, in which the bioinformatically provided candidates are never tested against biological reality. Another is the presence of alternative isoforms of proteins created by alternative splicing;[4] yet another is differential post-translational modification, such as differential glycosylation.

Applicants' invention makes possible the identification of the biologically relevant form of a protein biomarker; the prior art neither suggests nor motivates applicants' approach.

Applicants respectfully submit that the invention as claimed would have been nonobvious over the references of

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[4] Recent reports suggest that at least one-third, and likely a higher percentage, of human genes are alternatively spliced. Hanke *et al.*, *Trends Genet.* 15(1):389 - 390 (1999); Mironov *et al.*, *Genome Res.* 9:1288-93 (1999); Brett *et al.*, *FEBS Lett.* 474(1):83-6 (2000). With the Drosophila homolog of one human gene reported to have 38,000 potential alternatively spliced variants, Schmucker *et al.*, *Cell* 101:671 (2000), it now appears that alternative splicing is not only ubiquitous, but indeed may permit the human genome to encode millions, perhaps tens of millions, of structurally distinct proteins and protein isoforms.

record, individually or as combined, and that the rejection is thus in error and should be withdrawn.

Respectfully submitted,

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